abcam

Product datasheet

Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free ab215985

יובעדער RabMAb

★★★★★ 1 Abreviews 5 References 画像数 16

製品の概要

製品名 Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free

製品の詳細 Rabbit monoclonal [EPR3692] to HLA-DR - Low endotoxin, Azide free

由来種 Rabbit

特異性 Signal detected in rat sample is the ortholog of HLA.

アプリケーション 適用あり: Flow Cyt (Intra), Mass Cytometry, ICC/IF, IHC-P, WB

適用なし: №

種交差性 交差種: Rat, Human

交差が予測される動物種: Recombinant fragment 4 非交差種: Mouse

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HEK-293T cells transfected with a human HLA-DR expression vector containing a his-tag

whole cell lysate. Flow Cyt (intra): Raji cells. ICC/IF: Hut-78 cells. IMC: Human tonsil tissue IHC-P: Human tonsil tissue, human skin tissue, human spleen tissue, liver vessels tissue, skin vessels tissue, endometrial carcinoma vessels tissue, human kidney tissue, rat spleen tissue and rat colon

特記事項 ab215985 is the carrier-free version of ab92511.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Our <u>Low endotoxin, azide-free formats</u> have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

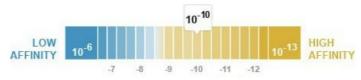
Mouse: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

解離定数(K_D 値) $K_D = 1.67 \times 10^{-10} M$



Learn more about K_D

バッファー pH: 7.20

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR3692

アイソタイプ IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab215985の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
Mass Cytometry		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 29 kDa.

追加情報

Is unsuitable for IP.

ターゲット情報

機能

Binds peptides derived from antigens that access the endocytic route of antigen presenting cells (APC) and presents them on the cell surface for recognition by the CD4 T-cells. The peptide binding cleft accommodates peptides of 10-30 residues. The peptides presented by MHC class II molecules are generated mostly by degradation of proteins that access the endocytic route, where they are processed by lysosomal proteases and other hydrolases. Exogenous antigens that have been endocytosed by the APC are thus readily available for presentation via MHC II molecules, and for this reason this antigen presentation pathway is usually referred to as exogenous. As membrane proteins on their way to degradation in lysosomes as part of their normal turn-over are also contained in the endosomal/lysosomal compartments, exogenous antigens must compete with those derived from endogenous components. Autophagy is also a source of endogenous peptides, autophagosomes constitutively fuse with MHC class II loading compartments. In addition to APCs, other cells of the gastrointestinal tract, such as epithelial cells, express MHC class II molecules and CD74 and act as APCs, which is an unusual trait of the GI tract. To produce a MHC class II molecule that presents an antigen, three MHC class II molecules (heterodimers of an alpha and a beta chain) associate with a CD74 trimer in the ER to form an heterononamer. Soon after the entry of this complex into the endosomal/lysosomal system where antigen processing occurs, CD74 undergoes a sequential degradation by various proteases, including CTSS and CTSL, leaving a small fragment termed CLIP (class-Il-associated invariant chain peptide). The removal of CLIP is facilitated by HLA-DM via direct binding to the alpha-beta-CLIP complex so that CLIP is released. HLA-DM stabilizes MHC class II molecules until primary high affinity antigenic peptides are bound. The MHC II molecule bound to a peptide is then transported to the cell membrane surface. In B-cells, the interaction between HLA-DM and MHC class II molecules is regulated by HLA-DO. Primary dendritic cells (DCs) also to express HLA-DO. Lysosomal miroenvironment has been implicated in the regulation of antigen loading into MHC II molecules, increased acidification produces increased proteolysis and efficient peptide loading.

配列類似性

Belongs to the MHC class II family.

Contains 1 lg-like C1-type (immunoglobulin-like) domain.

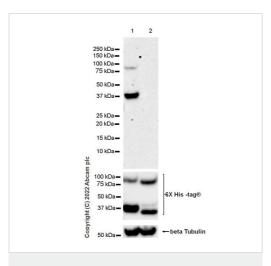
翻訳後修飾

Ubiquitinated by MARCH1 or MARCH8 at Lys-244 leading to down-regulation of MHC class II. When associated with ubiquitination of the beta subunit of HLA-DR: HLA-DRB4 'Lys-254', the down-regulation of MHC class II may be highly effective.

細胞内局在

Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus > trans-Golgi network membrane. Endosome membrane. Lysosome membrane. Late endosome membrane. The MHC class Il complex transits through a number of intracellular compartments in the endocytic pathway until it reaches the cell membrane for antigen presentation.

画像



Western blot - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)

All lanes : Anti-HLA-DR antibody [EPR3692] (<u>ab92511</u>) at 1/1000 dilution

Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with a human HLA-DR expression vector containing a his-tag, whole cell lysate

Lane 2: HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with a human HLA-DOA expression vector containing a his-tag, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

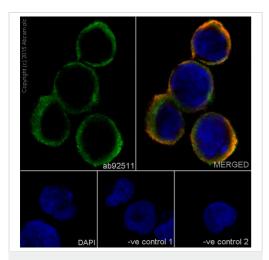
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 29 kDa Observed band size: 37 kDa

Exposure time: 3 minutes

 $\begin{tabular}{ll} \textbf{Blocking buffer and concentration}: 5\% \ NFDM/TBST \\ \begin{tabular}{ll} \textbf{Diluting buffer and concentration}: 5\% \ NFDM/TBST \\ \end{tabular}$

This antibody does not cross-react with human HLA-DOA.



Immunocytochemistry/ Immunofluorescence - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)

ab92511 staining HLA-DR in HuT-78 (human Sezary syndrome) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. ab7291 and ab150120 were used as counterstains for primary antibody ab92511 and secondary antibody ab150077 respectively and DAPI was used as a nuclear counterstain.

Negative control 1: Rabbit primary antibody and anti-mouse secondary antibody (<u>ab150120</u>)

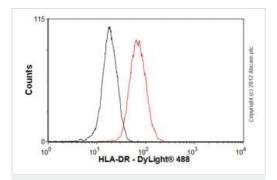
Negative control 2: Mouse primary antibody (<u>ab7291</u>) and antirabbit secondary antibody (<u>ab150077</u>)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

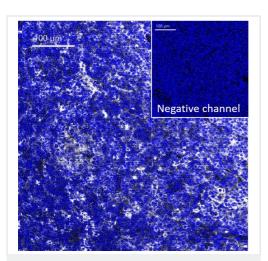
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labelling HLA-DR with <u>ab92511</u> at 1/10000 dilution (0.07 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody. Positive staining on human tonsil tissue. The section was incubated with <u>ab92511</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control.

Heat mediated antigen retrieval with Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 30 minutes.



Flow Cytometry (Intracellular) - Anti-HLA-DR antibody [EPR3692] - Low endotoxin, Azide free (ab215985)



Mass Cytometry - Anti-HLA-DR antibody [EPR3692]

- Low endotoxin, Azide free (ab215985)

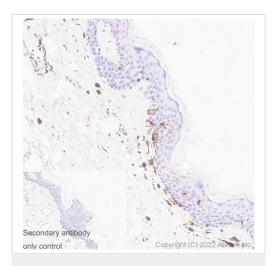
This image is courtesy of the Single Cell & Imaging Mass Cytometry Analysis Platform, Goodman Cancer Research Centre, McGill University

Overlay histogram showing Raji cells stained with <u>ab92511</u> (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (<u>ab92511</u>, 1/50) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-rabbit lgG (H+L) (<u>ab96899</u>) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

Imaging Mass Cytometry™ (IMC™) image of human tonsil tissue stained with Anti-HLA-DR antibody [EPR3692]. ab215985 (carrier-free antibody, purified) was metal-conjugated using a Maxpar® Antibody Labeling Kit from Fluidigm. Immunostaining was performed according to Fluidigm's protocols. Briefly, slides were subject to deparaffinization and heat-induced epitope retrieval, followed by overnight incubation at 4°C with an antibody cocktail containing metal-tagged antibodies in blocking buffer. Slides were subsequently washed with 0.2% Triton-X and 1x PBS, counterstained with Cell-ID™ Intercalator-Ir diluted at 1/400 in 1x PBS for 30 min at room temperature, rinsed for 5 min with distilled H2O, and air-dried prior to IMC™ acquisition. IMC™ acquisition was performed using the Fluidigm Hyperion™ Imaging System.

Imaging Mass Cytometry™, IMC™, Cell-ID™, Hyperion™ and Maxpar® are trademarks of Fluidigm Canada



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

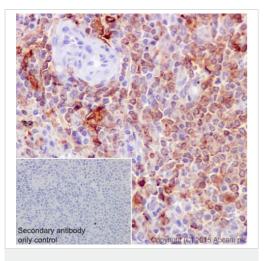
[EPR3692] - Low endotoxin, Azide free (ab215985)

Immunohistochemical analysis of paraffin-embedded human skin tissue labelling HLA-DR with <u>ab92511</u> at 1/10000 dilution (0.07 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) secondary antibody. Positive staining on human skin tissue. The section was incubated with <u>ab92511</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control.

Heat mediated antigen retrieval with Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 30 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)

<u>ab92511</u> staining HLA-DR in human spleen tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/700. A goat anti-rabbit IgG H&L (HRP) <u>ab97051</u> was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

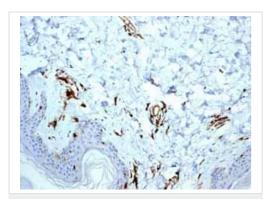


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)

<u>ab92511</u> showing positive staining in Normal liver vessels tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



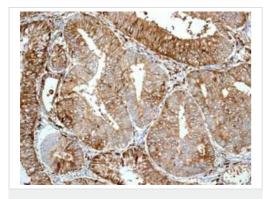
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)

ab92511 showing positive staining in Normal skin vessels tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).



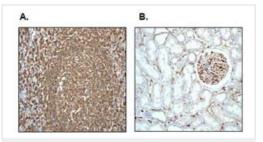
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)

<u>ab92511</u> showing positive staining in Endometrial carcinoma vessels tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

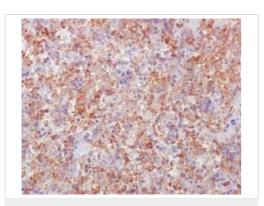
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)

ab92511, at a 1/100 dilution, staining HLA-DRA in paraffin embedded Human tonsil (A) and Human kidney (B) tissue by Immunohistochemistry. Detection: by DAB staining

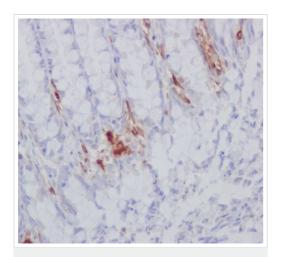


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling HLA DR with <u>ab92511</u> at a dilution of 1/1000. A Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) was used as the secondary antibody, at a dilution of 1/500. Counter stained with hematoxylin.

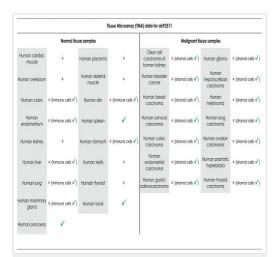
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

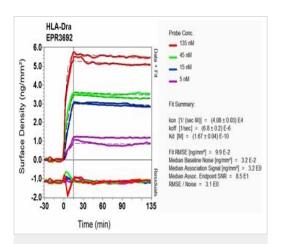
[EPR3692] - Low endotoxin, Azide free (ab215985)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling HLA DR with <u>ab92511</u> at a dilution of 1/1000. A Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) was used as the secondary antibody, at a dilution of 1/500. Counter stained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HLA-DR antibody

[EPR3692] - Low endotoxin, Azide free (ab215985)



OI-RD Scanning - Anti-HLA-DR antibody [EPR3692]

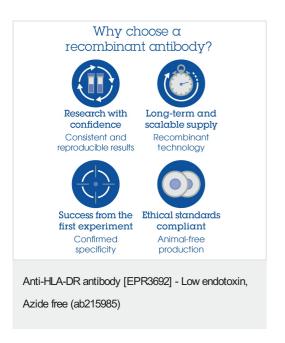
- Low endotoxin, Azide free (ab215985)

Tissue Microarrays stained for "Anti-HLA-DR antibody [EPR3692]" using "ab92511" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab92511 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92511).

Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about K_D



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